Sterile Insect Technique and Mediterranean Fruit Fly (Diptera: Tephritidae): Assessing the Utility of Aromatherapy in a Hawaiian Coffee Field

TODD E. SHELLY,[1,2,3] DONALD O. MCINNIS,[4] CHARLES RODD,[5] JAMES EDU,[1] AND ELAINE PAHIO[1]

J. Econ. Entomol. 100(2): 273–282 (2007)

ABSTRACT The sterile insect technique (SIT) is widely used in integrated programs against tephritid fruit fly pests, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Unfortunately, the mass-rearing procedures inherent to the SIT often lead to a reduction in the mating ability of the released males. One potential solution involves the prerelease exposure of males to particular attractants. In particular, exposure of male Mediterranean fruit flies to ginger, *Zingiber officinale* Roscoe, root oil (GRO) has been shown to increase mating success in laboratory and field cage trials. Here, we describe a field experiment that compares the level of egg sterility observed in two Hawaiian coffee, *Coffea arabica* L., plots, with GRO-exposed, sterile males released in one (treated) plot and nonexposed, sterile males released in the other (control) plot. Once per week in both plots over a 13-wk period, sterile males were released, trap captures were scored to estimate relative abundance of sterile and wild males, and coffee berries were collected and dissected in the laboratory to estimate the incidence of unhatched (sterile) eggs. Data on wild fly abundance and the natural rate of egg hatch also were collected in a remote area that received no sterile males. Despite that sterile:wild male ratios were significantly lower in the treated plot than in the control plot, the incidence of sterile eggs was significantly higher in the treated plot than in the control plot. Correspondingly, significantly higher values of Fried's competitiveness index (C) were found, on average, for treated than control sterile males. This study is the first to identify an association between the GRO "status" of sterile males and the incidence of egg sterility in the field and suggests that prerelease, GRO exposure may represent a simple and inexpensive means to increase the effectiveness of Mediterranean fruit fly SIT programs.

KEY WORDS Mediterranean fruit fly, sterile insect technique, aromatherapy

The sterile insect technique (SIT) is an environmentally benign approach for suppressing or eradicating insect pests, and it is widely used in integrated programs against tephritid fruit fly pests, particularly the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Hendrichs et al. 2002). The technique involves mass production of males of the target species and release of irradiated (sterilized) males into the environment. Matings between sterile males and wild females yield infertile eggs, thus reducing the reproductive potential of the wild population. To a large extent, the success of the SIT depends on the ability of released, sterile males to attract, and obtain matings, with wild females. This capability is especially important for species, such as *C. capitata*, in which females display a high degree of mate discrimination, based apparently on male courtship performance (Whittier et al. 1992, 1994).

Unfortunately, the mass-rearing procedures inherent to the SIT often lead to a reduction in the mating success of released *C. capitata* males (Rossler 1975, McInnis et al. 1996, Lance et al. 2000). Thus, a persistent and important challenge for Mediterranean fruit fly SIT is the development of simple and inexpensive means to enhance the mating ability of sterile males. In recent years, several studies have focused on two modifications to the prerelease environment of adult, sterile males that might enhance their performance in the field. One line of research has dealt with the composition of the adult diet, and, in particular, has examined whether supplementing the standard sugar agar diet with protein (yeast hydrolysate) enhances the mating frequency of sterile males. These studies have generated mixed results, e.g., supplementary protein boosted the mating performance of sterile males in some studies (Blay and Yuval 1997, Kaspi and Yuval 2000) but not in others (Shelly and Kennelly 2002, Shelly and McInnis 2003). Reasons for this dis-

[1] USDA–APHIS, 41-650 Ahiki St., Waimanalo, HI 96795.
[2] Center for Conservation Research and Training, University of Hawaii, Honolulu, HI 96822.
[3] Corresponding author, e-mail: todd.e.shelly@aphis.usda.gov.
[4] USDA–ARS-PBARC, 2727 Woodlawn Ave., Honolulu, HI 96822.
[5] USDA–ARS–PBARC, P.O. Box 1330, Kapaa, HI 96746.

crepancy are not known, and future work may yet identify those situations where dietary protein constitutes an effective means for improving the SIT.

A second set of studies has investigated the effect of the olfactory environment on the performance of sterile males. This research has shown that male exposure to the aroma of ginger, *Zingiber officinale* Roscoe, root oil (GRO), which contains the known male attractant α-copaene (Flath et al. 1994a,b), dramatically increases the mating success of sterile male Mediterranean fruit flies. This research has explored the applicability of this phenomenon to large-scale Mediterranean fruit fly SIT by progressively increasing the scale of male exposure and the rigor of the testing procedure. Initially, GRO exposure was conducted on small groups of males (25 individuals) held in small cups (volume 400 ml) (Shelly 2001). Subsequently, increased mating success was reported after GRO exposure involving ≈36,000 males held in storage boxes (so-called PARC boxes, 0.60 by 0.48 by 0.33 m [length by width by height]; Shelly et al. 2004a), ≈1.25 million males held in eclosion towers (0.76 by 0.76 by 1.5 m; Shelly et al. 2006), and ≈13 million males held in large trailers (17.8 by 3.05 by 2.4 m; T.E.S., unpublished data). Likewise, assessment of GRO exposure's effectiveness has been conducted under increasingly natural conditions. Initially, short-term (4-h) tests were conducted in laboratory cages (30 by 30 by 40 cm; Shelly and Kennelly 2002) or small ("single tree") field cages (3 m in height by 2.5 m in diameter; Shelly 2001, Shelly et al. 2004a). More recently, however, we have measured mating performance over several days in large field enclosures (16 by 6 by 2.5 m) containing multiple (>10 host trees; Shelly et al. 2005). Although the relative strength of the effect has varied among the different studies, GRO exposure of adult male Mediterranean fruit flies has consistently increased their mating success independently of the exposure or assessment protocol.

The goal of the current study was to assess the effectiveness of GRO exposure, a procedure termed aromatherapy (Shelly et al. 2004a), in a Hawaiian coffee, *Coffea arabica* L., field. As described below, we released GRO-exposed and nonexposed sterile males in separate plots and dissected coffee berries to determine the level of egg sterility induced by the released males. As shown below, the results were consistent with previous research, and, on average, a higher proportion of sterile eggs was found in the plot receiving GRO-exposed (treated) males than in the plot with nonexposed (control) males. Because this study represents the first test of aromatherapy conducted under open field conditions, we conclude that the results obtained provide support for the incorporation of prerelease GRO exposure as a standard protocol of Mediterranean fruit fly SIT.

Materials and Methods

Study Site. Fieldwork was conducted in a coffee field on the southeastern portion of Kauai Island, HI, September–December 2003. One plot was established at each end of a dumb-bell shaped gulch, with the northern end (3.8 ha) being designated the control plot (i.e., receiving control sterile males) and the southern end (3.9 ha) being designated the treated plot (i.e., receiving GRO-treated sterile males). The intervening strip of land, which was 1 km in length and also planted in coffee, was separated from the northern plot by a 20–30-m-wide area of nonhost plants, but it was contiguous with the southern plot. The gulch has an elevation of ≈200 m and receives ≈75 mm of rainfall per yr, mostly in the winter (December–March). In addition to the study plots, we also collected coffee berries from a field ≈3 km from the gulch to assess natural levels of egg hatch. This "remote" area had similar environmental conditions as the gulch but received no releases of sterile males.

Sterile Insects: Production, Handling, and Release. Mass-reared flies were from a genetic sexing strain (Vienna-7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu, HI. The strain possesses a temperature sensitive lethal (*tsl*) mutation, such that treating the eggs with high temperature kills all female zygotes, thereby allowing production of males exclusively (Franz et al. 1996). Larvae were reared on a standard diet (Tanaka et al. 1969), and pupae were dyed (pink on all but one date; see below) and irradiated 2 d before eclosion under hypoxia at 150 Gy of gamma irradiation from a ^{137}Cs source. After irradiation, the pupae (contained in six plastic bags each containing ≈190,000 individuals) were stored overnight at 15°C and air shipped (once per week) to Kauai (20-min flight) the next morning.

Upon their arrival in Kauai, the pupae were transported to the USDA–ARS laboratory in Kapaa. Following the protocol of the California SIT program, we placed 100 ml of pupae in paper bags (1 ml is ≈60 pupae), and six bags were placed in individual PARC boxes. Pupae were placed in a total of 30 PARC boxes, and 15 boxes were stored in each of two separate rooms (24–26°C, 50–90% RH and a photoperiod of 12:12 [L:D] h of artificial light). One room was assigned for control males throughout the entire study, whereas the other room was assigned for treated males. Most adult emergence occurred 2 d after pupal placement, and emerging sterile males were fed a sugar-agar gel placed on the screened opening on top of the box.

For GRO exposure, we placed 1 ml of GRO on blotter paper (4-cm square) and placed the paper on the screened opening of all the PARC boxes assigned to the treated category (the GRO was obtained from Citrus & Allied Essences Ltd., Lake Success, NY, and contains 0.4% α-copaene by volume, T. W. Phillips, personal communication). The exposure period lasted 24 h starting at 0600 hours on the third day after peak emergence and ending on the day of release (i.e., sterile males were 4 d old when released).

Flies were released once a week between 22 September and 15 December 2003 (13 releases). Control and treated males were transported in separate vehicles to their respective release plots (≈30 min drive

from the Kapaa facility). In each plot, we designated 15 evenly spaced release points, which were used throughout the entire study, and released males from one PARC box at each of these points. Boxes were opened and set on the ground, and males flew off on their own volition. After 20–30 min, the boxes were shaken to remove the remaining flies. Owing to the high abundance of the wild population, the release of ≈540,000 sterile males (≈36,000 males per box, 15 boxes) per study plot yielded very low sterile:wild male ratios (nearly all values <1; see below). Although at least moderate overflooding (ratios >10) may have been desirable, our release numbers were constrained because most of the pupae produced by the Waimanalo rearing facility were shipped to California for use in a SIT program and were thus unavailable for our study.

Monitoring Adult Populations. To estimate the relative abundance of sterile and wild males, we placed five, evenly spaced male traps in each study plot. Traps were lidded, plastic cups (950 ml in volume) with four circular openings (2.5 cm in diameter) on opposite sides of the cup to allow fly entry. Each trap contained one plug impregnated with 2 g of trimedlure (a male attractant) and a toxicant (VaporTape, Hercon Environmental, Emigsville, PA). Captured flies were collected and the lure and toxicant were replaced weekly. Because of the large number of males captured, released and wild males were counted in a 0.5-g (dry weight) subsample (≈400 flies) for each trap, and the total numbers were estimated by extrapolating to the total weight of the trap catch for the individual traps. Sterile (with dye) and wild (without dye) were identified using a black (UV) light. In addition to the traps placed within the gulch, we ran three traps per week in the remote area to assess population trends of wild flies in an area that did not receive any releases of sterile males. Abundance estimates for wild males in the remote area were made following the procedure described above.

The traps also were used to determine whether sterile males moved between the study plots, thus potentially confounding data interpretation. On one release date (1 December), we released control and treated males dyed with different colors (blue and yellow, respectively) and then checked for the appearance of a "mismatched" male (i.e., a blue-dyed male in the treated plot or a yellow-dyed male in the control plot) in the traps the following week. No such mismatch was detected in either plot; consequently, we concluded that there was little, or no, movement of sterile males between the study plots.

Measuring Egg Sterility. Ripe berries were collected once a week from each of the experimental plots as well from the remote site. In the plots, berries were collected in the vicinity (within 5–10 m) of the five trap sites to ensure an even spatial distribution of fruit sampling. Approximately 100 berries were collected per site (i.e., 500 per plot) and held separately in paper bags. In the remote locality, 200–300 berries were collected haphazardly over an area of roughly 100–200 m^2. The fruit samples were transported to Oahu by air on the same day as collection and stored overnight at 15°C. Berries were dissected the next day under a dissecting microscope, and we recorded the number of hatched and unhatched eggs and larvae found per fruit. Unhatched eggs were placed on moistened blotter paper and held at 27°C for 48 h. After incubation, we examined the eggs under a dissecting microscope and tallied the total number of hatched and unhatched eggs.

For a fruit, we estimated the number of hatched (fertile) individuals as the sum of the number of hatched eggs or larvae found in the fruit (using the larger value of the two values) plus the number of eggs that hatched after the incubation procedure. Eggs that did not hatch during the incubation period were classified as sterile. For each experimental plot, we dissected 20 fruits per sampling area (i.e., a total of 100 fruit per plot per sampling date), including only fruits that contained at least one egg or larva. One hundred fruit from the remote area also were dissected. We recognize that our operational definition of egg sterility may have included fertilized eggs that, for some reason, were unviable. However, as shown below, egg hatch from the remote area was quite high, suggesting mortality was a minor cause of nonhatching.

Calculation of Fried's Competitiveness (*C*) Index. For each sampling area within each experimental plot and for each sampling date, we computed the competitiveness index (*C*) (Fried 1971) to compare the performance of control and treated sterile males versus wild males, where $C = (W/S) \times [(H_w - H_c)/(H_c - H_s)]$, where W is number of wild males recorded in the trap, S is number of sterile males recorded in the trap, H_w is percentage of fertile individuals found in fruit collected in remote area (thus providing a baseline value for the incidence of egg sterility within a wild population), H_c is percentage of fertile individuals recorded from fruit collected in the sampling area, and H_s is percentage of egg hatch from wild females after mating with sterile males (this variable was assigned the value of 0.3% based on Shelly et al. [2005b]). Note that *C* values were computed using trap catch data from the same week as well as from 1 or 2 wk preceding a given fruit collection. We included these latter computations to accommodate a potential delay between mating and oviposition, which would reduce the temporal correspondence between egg hatch data and the ratio of sterile:wild males at the time of mating. A competitive index of one indicates equal competitive ability between sterile and wild males. When $C < 1$, sterile males are competitively inferior to wild males, and when $C > 1$, sterile males are more competitive than wild males. It should be noted that the *C* values presented are best considered imprecise indicators of true competitiveness, because their computation relied on field measurements of sterile:wild male ratios, which themselves were quite variable (Fig. 1). Thus, their usefulness derives from the illustration of general trends (i.e., consistent differences between control and treated *tsl* males) rather than the absolute, numerical values associated with the *C* index.

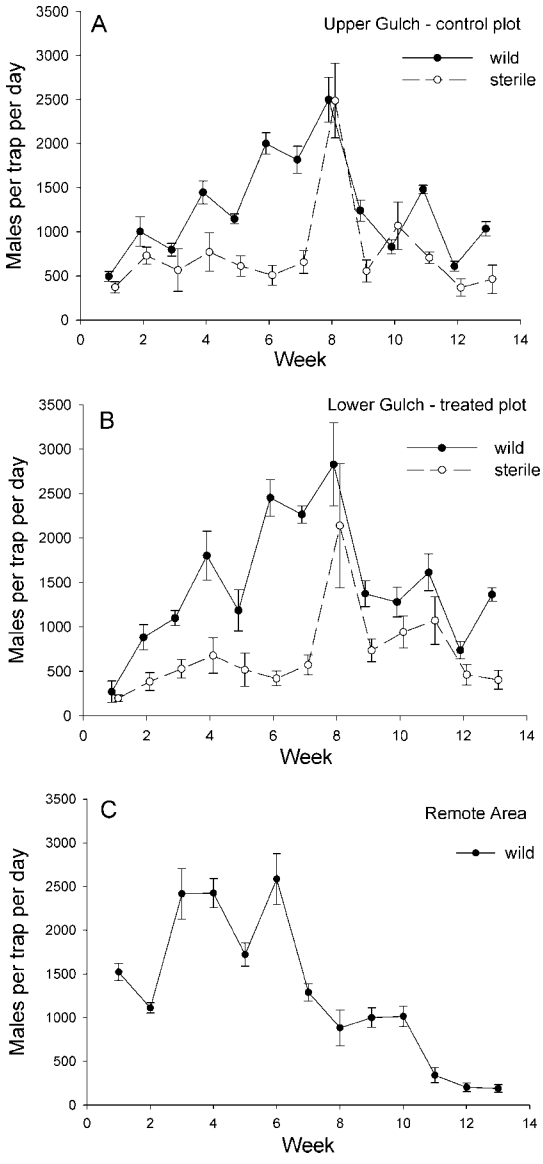

Fig. 1. Relative abundance of wild and sterile male Mediterranean fruit flies in the control (A) and treated (B) gulch plots and wild males in the remote plot (C) over the study period. Values represent the estimated average ± SE number of males captured per trap per day per location. Weekly counts were made for five traps in each of the gulch plots and three traps in the remote area.

Statistical Analyses. Control and treated plots were compared with respect to the absolute and relative numbers of trapped wild and sterile males, number of eggs and larvae dissected from collected fruit, percentage of sterile eggs, and *C* values. For all factors except *C* values, parametric assumptions of normality and equal variance were met (data on trap captures were normalized using a $\log_{10}$ transformation); consequently, interplot comparisons were made with 1) a *t*-test using data from all the sampling areas over all sampling dates ($n = 5$ sampling areas per plot × 13 sampling dates = 65 values per plot) and 2) a paired *t*-test using mean values computed for the respective study plots across sampling dates ($n = 1$ mean per plot per sampling date × 13 sampling dates = 13 values per plot). In these tests, proportions (overflooding ratios and relative abundance of sterile eggs) were arcsine transformed for analysis. Means ± 1 SE are presented. *C* values were not normally distributed; hence, we used the Mann–Whitney *U*-test when comparing data from all sampling areas over all sampling dates and the Wilcoxon signed rank test for the pairwise comparisons of means across sampling dates. In a few instances, computed *C* values were negative (i.e., percentage of egg sterility was higher in the remote area than in an area receiving sterile males), and these data were assigned a value of zero for statistical analysis.

As evident from the preceding discussion, the scale of the project limited our field work and subsequent data analysis to two plots only (plus the remote area). In 2004, we planned to repeat the experiment, using the same two plots but reversing the treatments, but heavy rains blocked access to the study plots for several weeks and prevented systematic releases of sterile males. Limited to multiple measurements of two plots, the analyses used necessarily relied on the assumption that the measurements made within plots were, in fact, statistically independent (as noted above, fly movement between the two plots seemed unlikely, suggesting statistical independence in the measurements between plots).

To test for independence through time, we tested for serial correlation (using the parametric mean square successive difference test, test statistic C_{SD}; Zar 1996) of the absolute numbers of wild and sterile males as well as the overflooding ratio for each study plot (using weekly averages based on the five sampling areas) and for each of the sampling areas within each study plot. As shown below, the abundance of wild males showed a distinct, and similar, temporal pattern in both plots; thus, the sequence of wild male numbers was significantly nonrandom for the treated plot ($C_{SD} = 0.46$, $n = 13$, $P < 0.05$) and "marginally" nonrandom for the control plots ($C_{SD} = 0.36$, $n = 13$, $P < 0.1$). However, when examined for the individual sampling areas within the plots, the data showed more randomness: estimates of wild male abundance showed nonrandom temporal distribution for only two sampling sites in the treated plot and only one sampling site in the control plot. Analyses of the abundance of sterile males or the overflooding ratios failed to detect any temporal association of values when performed for each plot or for each sampling area within each plot. Based on these results, we concluded that, although there was evidence of temporal correlation of wild fly numbers (at the level of the entire plot), this simply reflects the natural trend in population size through the coffee fruiting season and that temporal randomness in both the abundance of sterile males and the overflooding ratios (for both plots and all sampling areas therein) implies that any measurable impact of sterile male releases on egg sterility was

not a statistical artifact stemming from some temporal association in the abundance estimates of sterile males. In addition, field data from other studies (Wong et al. 1982, Baker and Chan 1991, Shelly et al. 1994, McInnis et al. 2002) indicate that the majority of sterile males survive for only a few days after release, further suggesting temporal independence of measurements made on successive sampling dates.

Regarding independence in space, although we did not collect data on within-plot movement by either wild or sterile flies, other studies have shown that Mediterranean fruit fly adults show low dispersal. Whereas released sterile males have been detected >1 km from the release site (Severin and Hartung 1912, Katiyar and Valerio 1963), it seems that the majority of released males move only short distances. For example, Baker et al. (1986) estimated dispersion of sterile males in a coffee field and found that the mean distance traveled was only 5–10 m per day and correspondingly that, even 5 d after release, the mean distance traveled was 40–50 m (also see Baker and Chan 1991). Likewise, working in a mixed fruit orchard, Wong et al. (1982) found that ≈75% of sterile males were captured within 60 m of the release point. Because our release points were all separated by >50 m, these data suggest that, although some movement undoubtedly occurred between release sites within a plot, sterile males had greatest impact in the immediate vicinity of their release point.

Results

Trap Captures of Wild and Sterile Males. Wild flies were extremely abundant in both gulch plots (Fig. 1). Over the entire study period, the average number of wild males captured per trap per day was 1,260 ± 76 in the control plot and 1,511 ± 94 in the treated plot. There was no significant difference between these means using a *t*-test comparing all trap captures between the plots ($t = 1.8$, df = 128, $P = 0.07$) or a paired *t*-test comparing mean trap captures in the two plots over the individual sampling dates ($t = 1.6$, df = 12, $P = 0.13$). The same temporal pattern was observed in captures of wild males in the two gulch study plots, with numbers increasing to peak values in weeks 6–8 (late October–early November) and then decreasing toward the end of the study period. Large numbers of wild males also were captured in the remote area (mean = 1,279 ± 133 males per trap per day, $n = 3$ traps per date × 13 trap dates = 39 values). Although temporal variation in trap captures followed the same overall pattern as in the gulch plots, numbers of wild males peaked earlier (weeks 3–6, early to late October) and correspondingly declined earlier than in the gulch plots.

On nearly all sampling dates, sterile males were captured in much lower numbers than wild males in both control and treated plots (Fig. 1). Over the entire study period, the average number of sterile males captured per trap per day was 758 ± 81 in the control plot and 695 ± 84 in the treated plot. There was no significant difference between these means using a *t*-test comparing all trap captures between the plots ($t = 0.9$, df = 128, $P = 0.39$) or a paired *t*-test comparing mean trap captures over the individual sampling dates ($t = 1.3$, df = 12, $P = 0.22$). Aside from a spike in week 8, average trap captures of sterile males were relatively constant (400–600 males per trap per day) over the entire study period in both control and treated plots.

Owing to the high numbers of wild flies, overflooding (sterile:wild males >1) was rarely achieved during the study. Ratios of sterile:wild males computed for the individual sampling dates (pooling data from the five traps per plot) averaged 0.62 ± 0.08 ($n = 13$) and exceeded one on only one date (week 10) in the control plot and averaged 0.50 ± 0.05 ($n = 13$) and never exceeded one in the treated plot. Sterile:wild male ratios observed in the control plot were, on average, significantly larger in those observed for the treated plot by using a *t*-test comparing values computed for all trap collections ($t = 2.1$, df = 128, $P = 0.037$) or a paired *t*-test comparing mean sterile:wild male ratios over the individual sampling dates ($t = 2.4$, df = 12, $P = 0.03$).

Given the low sterile:wild male ratios in the gulch plots, along with the November–December decline in wild male abundance in the remote area, it seems unlikely that the releases of sterile males were responsible for the late-season decrease in wild male abundance in the gulch plots. Consequently, it is important to recognize that the impact of released, sterile males, and, potentially, the additional influence of GRO exposure, would be discernible, not through any reduction of the wild fly population, but through the increased incidence of egg sterility.

Yield from Fruit Dissections. Fig. 2 provides data on the sample sizes used to compute percentage of egg sterility and competitiveness for the control and treated plots. In both plots, the number of individuals observed in fruit was uniformly low during weeks 1–7 but then increased substantially during the latter weeks of the study. There were no significant differences observed between the control and treated plots in the number of individuals observed (larvae/hatched eggs and unhatched eggs combined) per sampling area during weeks 1–7, weeks 8–13, or over the entire study period by using data from individual sampling areas in *t*-tests (weeks 1–7: $t = 1.6$, df = 68, $P = 0.10$; weeks 8–13: $t = 1.8$, df = 58, $P = 0.09$; and weeks 1–13: $t = 1.5$, df = 128, $P = 0.13$). Likewise, in paired *t*-tests comparing mean numbers of individuals observed per sampling area over individual sampling dates, there was no significant difference between control and treated plots for weeks 8–13 ($t = 2.0$, df = 5, $P = 0.10$) or weeks 1–13 ($t = 2.0$, df = 12, $P = 0.06$). The number of individuals observed in fruits was, however, significantly greater in the treated plot than the control plot for weeks 1–7 ($t = 4.1$, df = 6, $P = 0.01$). This final result notwithstanding, the comparisons show that the overall sample sizes were similar for the two study plots.

Fruit collections were made independently of the three trapping (sampling) sites in the remote area,

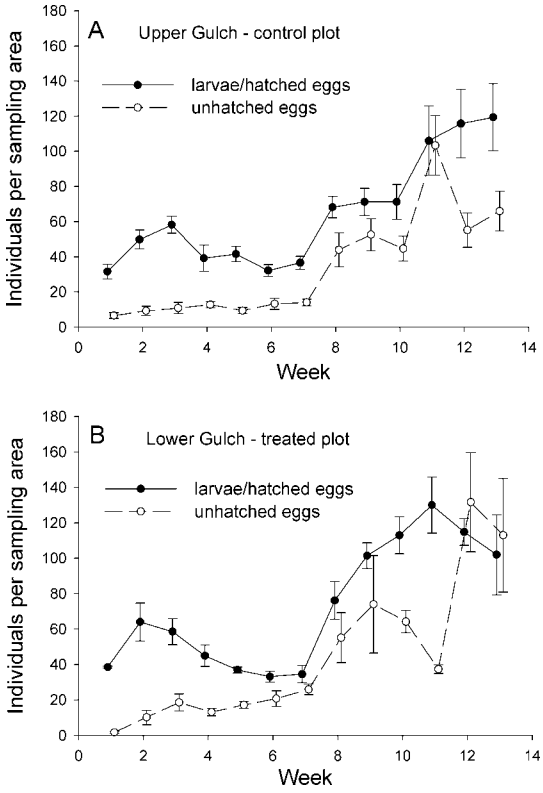

Fig. 2. Number of individuals (larvae/hatched eggs or unhatched eggs; the latter were subsequently incubated and scored for egg hatch) collected from coffee fruits from sampling areas within the control (A) and treated (B) gulch plots over the study period. Values represent means ± SE per sampling area; 20 fruit (containing ≥1 individual) were dissected per sampling area per week. Because each plot contained five sampling areas, five times the sum of the two curves gives the total number of individuals recorded per plot per week.

yielding a single total number of individuals observed per sampling date. Among sampling dates, the number of larvae/hatched eggs observed varied between 146 and 296, the number of unhatched eggs observed (and subsequently incubated) varied between 24 and 88, and the total number of individuals observed ranged from 170 to 351.

Egg Sterility Levels. Over the entire study period, the proportion of sterile eggs varied from 1 to 22% in the control plot and from 1 to 38% in the treated plot, with the lowest values in each plot observed during the initial 5 wk of the study (Fig. 3). Based on data from all sampling areas, mean levels of egg sterility were as follows: weeks 1–5, 3.0% (0.6) and 7.8% (1.2) for the control and treated plots, respectively ($t = 2.9$, $df = 48$, $P = 0.005$); weeks 6–13, 16.7% (1.4) and 23.9% (1.9) for the control and treated plots, respectively ($t = 2.8$, $df = 78$, $P = 0.03$); and weeks 1–13, 11.4% (1.9) and 17.1% (1.6) for control and treated plots, respectively ($t = 2.8$, $df = 128$, $P = 0.005$). Comparing mean plot values across sampling dates also revealed signif-

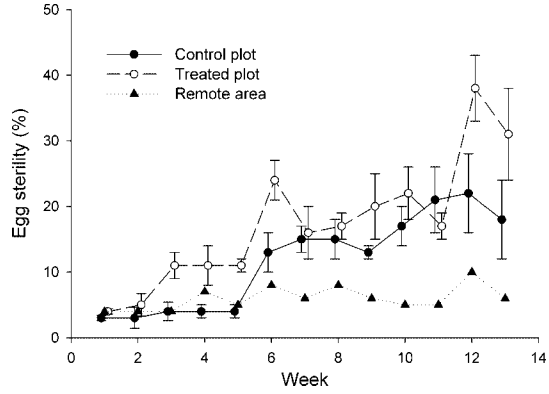

Fig. 3. Levels of egg sterility observed in control and treated plots and the remote area over the study period. Values for the two plots are weekly means ± SE based on dissections of 20 infested fruit per sampling area per plot. Values for the remote area are based on a single collection of 100 fruit per week.

icant interplot differences for weeks 6–13 ($t = 2.8$, $df = 7$, $P = 0.03$) and weeks 1–13 ($t = 3.7$, $df = 12$, $P = 0.003$). Because of the small sample sizes, this comparison did not show a difference between control and treated plots during the initial 5 wk ($t = 2.6$, $df = 4$, $P = 0.06$).

Levels of egg sterility were uniformly low across all sampling dates for the remote site (Fig. 3). Over all dates, the average proportion of sterile eggs was only 6.5 ± 0.50% (range 4–10%).

Competitiveness Values. Regardless of the computational method, competitiveness values were significantly higher for treated than control sterile males both when comparing data from all sampling area and dates as well as mean values across sampling dates (Fig. 4). When computed without a time lag, the average C value over the entire study was 0.42 (range 0–6.7) for the treated males and 0.17 (range 0–1.3) for the control males ($T = 4952.0$, $n_1 = n_2 = 65$, $P < 0.001$). Similarly, the paired sample comparison of means over individual sampling dates revealed a significant difference between treated and control plots ($W = 69.0$, $n_1 = n_2 = 13$, $P = 0.01$). When computed with a 1-wk time lag, the average C value over the entire study was 0.40 (range 0–5.7) for the treated males and 0.15 (range 0–1.1) for the control males ($T = 2913.5$, $n_1 = n_2 = 60$, smaller samples reflect 1-wk delay in computing C values, $P < 0.001$). Likewise, the paired-sample comparison of means over individual sampling dates revealed a significant difference between treated and control plots ($W = 78.0$, $n_1 = n_2 = 12$, $P < 0.001$). When computed with a 2-wk time lag, the average C value over the entire study was 0.65 (range 0–9.7) for the treated males and 0.22 (range 0–1.7) for the control males ($T = 3628.5$, $n_1 = n_2 = 55$, smaller samples reflect 2-wk delay in computing C values; $P < 0.001$). Likewise, the paired-sample comparison of means over individual sampling dates revealed a significant difference between treated and control plots ($W = 58.0$, $n_1 = n_2 = 11$, $P < 0.05$).

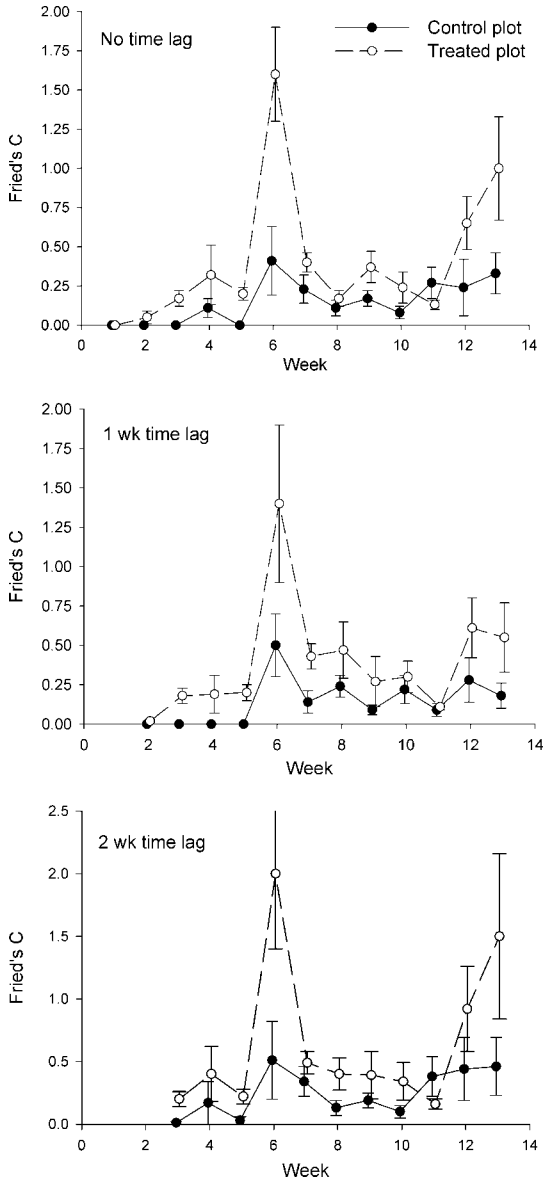

Fig. 4. Values of Fried's *C* for control and treated sterile males calculated with no time lag (i.e., data for egg sterility, male abundance, natural hatch [remote area] were all from the same sampling date) or with a 1- or 2-wk time lag. For the time lag plots, data for male abundance and natural hatch [remote area] were from the sampling date occurring one or 2 wk before the date from which egg sterility data were obtained). Values are weekly means ± SE based on trap captures and egg sterility estimates for five sampling areas per plot. See text for method of calculation.

It is noteworthy that the differences in mean values described in the preceding paragraph do not reflect a few aberrantly high or low *C* values but reflect consistent differences between control and treated *tsl* males. Over all 13 wk of the study, mean *C* values for treated males exceeded those for control males for 11 wk in calculations involving no time lag or a 1-wk time lag, respectively, and for 12 wk in calculations involving a 2-wk time lag (Fig. 4). Thus, although the imprecision of the computed *C* values is acknowledged, a consistent difference in relative magnitude was apparent between control and treated *tsl* males trend over the entire duration of the study.

Discussion

Previous studies have demonstrated that GRO exposure to sterile male Mediterranean fruit flies increases mating success in field cages (Shelly and McInnis 2001, Shelly et al. 2004a) and egg sterility levels in large field enclosures (Shelly et al. 2005). However, the current study provides the first data from the open field indicating that prerelease exposure of sterile males to GRO can increase the effectiveness of Mediterranean fruit fly SIT. Despite the finding that GRO-exposed, sterile males had lower abundance relative to wild males than did nonexposed, sterile males, the treated males induced higher levels of egg sterility than did their nonexposed counterparts. Consequently, estimates of male mating competitiveness, based on Fried's index, were significantly greater for GRO-exposed males than control, nonexposed males.

Relative to the sterile:wild male ratios achieved, the sterility levels reported in the current study are much higher than reported in other studies. Although wild males were usually more abundant than sterile males, egg sterility was, on average, 11% for the control plot and 17% for the treated plot. In contrast, in a study conducted in Guatemalan coffee fields using the same *tsl* strain, Rendon et al. (2004) found that egg sterility was <5% for overflooding ratios as high as 50:1. This finding likely derives from, in the current study, releasing sterile males directly from the ground into a small area containing a large population of wild flies. In this situation, sterile males were not required to disperse long distances in search of mating aggregations (leks) and presumably had a high probability of encountering wild females. In contrast, in the Guatemalan project, releases were made exclusively from aircraft over a much larger area (36 km^2) containing a much lower density of wild flies, and sterile males likely had a lower probability of encountering potential mates.

Although there was no increase in either the numbers of sterile males captured in the trimedlure traps or the overflooding ratios through time, levels of egg sterility were higher toward the end of the study for both control and treated plots. Other studies (McInnis et al. 1994; Rendon et al. 2004) similarly report increases in egg sterility with time since initiation of sterile male releases. This general pattern presumably reflects the temporal accumulation of wild females that mated with sterile males. Data on field longevity are unavailable, but based on observations (T.E.S., unpublished data) made in large field enclosures, wild females are likely to live, and oviposit, for several weeks. Consequently, the impact of sterile males may be evident only after an initial delay, because 1) rel-

atively few females have mated with sterile males during the initial period the start of releases, and, just as importantly, 2) there is no carryover of already mated females into this initial period.

The relatively high sterility levels reported here also contributed to the relatively high *C* values obtained. Over the entire study, we computed average *C* values of 0.17 and 0.42 for the nonexposed and GRO-exposed sterile males, respectively, whereas *C* values from other studies are generally much lower (computed averages: 0.01, Rhode et al. 1971 [Tables 1 and 2 for October 1968–February 1969]; 0.10, Wong et al. 1986 [Table 1; Fig. 1, 6 April 1981–2 June 1982]; 0.0002–0.001, McInnis et al. 1994, [Tables 2 and 3, 19 September–28 November]; and 0.0001–0.001, Rendon et al. 2004 [Fig. 7, weeks 21–37]). Perhaps more importantly, however, these low *C* values also reflected the high overflooding ratios achieved in these other studies. As noted previously, the overflooding ratio is used in computing *C*, such that, for a given level of egg sterility, higher overflooding ratios yield lower *C* values. In the current study, the numbers of sterile males rarely exceeded those of wild males, whereas overflooding ratios averaged >100:1 in the other studies and even averaged >1,000:1 in several cases (average overflooding ratios calculated using data from same tables and figures noted above; 2,938, Rhode et al. 1971; 165, Wong et al. 1986; 342–817, McInnis et al. 1994; and 410–520, Rendon et al. 2004).

It should be noted that the lower *C* values obtained in these other studies do not reflect lessened mating ability of sterile males, per se, but rather the numerical consequence that, as overflooding ratios become large, a decreasing proportion of released sterile males will actually encounter a wild female and have the opportunity to mate. Thus, the value of Fried's *C* as an indicator of the mating ability of sterile males varies inversely with the overflooding ratio used in its computation. Because of the low sterile:wild male ratios obtained in the current study, the *C* values computed more closely mirror the mating ability of GRO-exposed and nonexposed males as measured in field-cage trials. With equal numbers of sterile and wild males (75 males of each type) competing for 75 wild females in field cages, Shelly et al. (2004a) found that, on average, GRO-exposed males achieved 54% of the total matings, representing a *C* value of 1.17 (=54/46) compared with a *C* value of 0.42 in the field, and nonexposed males achieved 25% of all matings, representing a *C* value of 0.33 (=25/75) compared with a *C* value of 0.17 in the field. For both GRO exposed and nonexposed males, the *C* values obtained in field cages were approximately twice the values computed for the Kauai coffee field, probably reflecting the proximity of the sexes in the field cages. This effect notwithstanding, the approximate correspondence between cage and field *C* values show that the mating ability of sterile males (relative to wild males) is best measured under relatively low densities using equal numbers of sterile and wild males.

A major shortcoming of this project was our inability to demonstrate that the incorporation of GRO exposure in the release protocol actually increased the likelihood of eradication and/or reduced the time necessary to achieve eradication. The wild population was extremely abundant, with trap catches regularly exceeding 1,000 wild males per trap per day and with some values as high as 2,500–3,000 males per trap per day. These estimates are among the highest ever recorded for wild Mediterranean fruit fly populations (Rhode et al. 1971, Cheikh et al. 1975, Wong et al. 1986, McInnis et al. 1994, Rendon et al. 2004) and effectively precluded demonstration of a populational effect arising from our releases. As noted, the late-season decline in the wild population in the remote area indicates that the sterile male releases did not cause the contemporaneous decreases observed for the wild population in the gulch plots (Fig. 1). Given the extremely high density of wild flies in our study area, final proof regarding the utility of GRO exposure requires a comparable field experiment in a region with far fewer wild flies.

This future requirement notwithstanding, a substantial amount of evidence now exists suggesting that GRO aromatherapy is feasible on a programmatic scale and will improve the effectiveness of Mediterranean fruit fly SIT control programs. The chief points include the following: 1) GRO exposure increases mating competitiveness of sterile males (Shelly et al. 2004a); 2) GRO exposure increases the level of egg sterility in field environments (Shelly et al. 2005, this study); 3) with GRO exposure, lower overflooding ratios (i.e., release numbers) are required to achieve a given level of egg sterility (Shelly et al. 2005); 4) females mated initially to wild males are more likely to remate with GRO-exposed, sterile males (thus allowing for last male advantage in sperm competition, Saul and McCombs 1993) than nonexposed, sterile males (Shelly et al. 2004b); 5) under field conditions, the survival of sterile males exposed to GRO is similar to that of nonexposed males (Shelly et al. 2004a); 6) after aerial release, dispersal and survival are similar for GRO-exposed and nonexposed sterile males (Shelly et al. 2006); 7) at eclosion facilities, effective GRO exposure can be achieved for entire trailers (holding ≈13 million males; T.E.S., unpublished data) or eclosion towers (holding ≈1.25 million males; Shelly et al. 2005a) at a cost of approximately $0.05–0.15 per million males. Based on this information, two SIT-based control programs (in California and Florida, respectively) have already incorporated GRO exposure in their standard operating protocol.

From a programmatic perspective, the chief potential benefit of GRO aromatherapy is that, owing to enhanced male mating ability, it may allow reduction in the number of sterile males required to achieve eradication. Although use of genetic sexing strains has improved the field efficacy of Mediterranean fruit fly SIT, exceedingly large overflooding ratios are apparently still needed for effective control (McInnis et al. 1994, Rendon et al. 2004). For example, in the aforementioned study in Guatemalan coffee fields, Rendon et al. (2004) found that, even with a male-only strain, a substantial increase in egg sterility was not evident

until overflooding ratios >100:1 were realized. In that study, the maximum egg sterility observed was only 75%, and this was not recorded until an overflooding ratio of ≈800:1 was attained. Thus, although male-only strains improve Mediterranean fruit fly SIT compared with bisexual strains, the poor mating capability of the released males severely limits their effectiveness. Realizing the full benefits of male-only releases therefore will require modification of current mass rearing procedures to improve the mating ability of released sterile males.

Acknowledgments

We thank McBride Coffee for permission to work on their land and the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility for supplying the sterile males. We are also grateful to Chuck Brinkmann for assisting with releases, Russell Ijima for administrative and technical help, Jason Komatsu for assisting with fruit dissections, and Mindy Teruya for help with scoring hatch of incubated eggs.

References Cited

Baker, P. S., and A.S.T. Chan. 1991. Quantification of tephritid fruit fly dispersal: guidelines for a sterile releases programme. J. Appl. Entomol. 112: 410–421.

Baker, P. S., A.S.T. Chan, and M. A. Jimeno Zavala. 1986. Dispersal and orientation of sterile *Ceratitis capitata* and *Anastrepha ludens* (Tephritidae) in Chiapas, Mexico. J. Appl. Ecol. 23: 27–38.

Blay, S., and B. Yuval. 1997. Nutritional correlates to reproductive success of male Mediterranean fruit flies. Anim. Behav. 54: 59–66.

Cheikh, M., J. F. Howell, E. J. Harris, H. Ben Salah, and F. Soria. 1975. Suppression of the Mediterranean fruit fly in Tunisia with released sterile insects. J. Econ. Entomol. 68: 237–243.

Flath, R. A., R. T. Cunningham, T. R. Mon, and J. O. John. 1994a. Additional male Mediterranean fruit fly (*Ceratitis capitata* Wied.) attractants from angelica seed oil (*Angelica archangelica* L.). J. Chem. Ecol. 20: 1969–1984.

Flath, R. A., R. T. Cunningham, T. R. Mon, and J. O. John. 1994b. Male lures for Mediterranean fruit fly (*Ceratitis capitata* Wied.): structural analogues of α-copaene. J. Chem. Ecol. 20: 2595–2609.

Franz, G., P. Kerremans, P. Rendon, and J. Hendrichs. 1996. Development and application of genetic sexing systems for the Mediterranean fruit fly based on a temperature sensitive lethal, pp. 185–191. *In* B. A. McPheron and G. J. Steck [eds.], Fruit fly pests: a world assessment of their biology and management. St. Lucie Press, Delray Beach, FL.

Fried, M. 1971. Determination of sterile-insect competitiveness. J. Econ. Entomol. 64: 869–872.

Hendrichs, J., G. Franz, and P. Rendon. 2002. Medfly areawide sterile insect technique programmes for prevention, suppression, or eradication: the importance of mating behavior. Fla. Entomol. 85: 1–13.

Kaspi, R., and B. Yuval. 2000. Post-teneral protein feeding improves sexual competitiveness but reduces longevity of mass reared sterile male Mediterranean fruit flies. Ann. Entomol. Soc. Am. 93: 949–955.

Katiyar, K. P., and J. Valerio. 1963. Estudios sobre la dispersion y longevidad de la mosca del Mediterraneo *Ceratitis capitata* Wied., marcada con P^{32}. Turrialba 13: 181–184.

Lance, D. R., D. O. McInnis, P. Rendon, and C. G. Jackson. 2000. Courtship among sterile and wild *Ceratitis capitata* (Diptera: Tephritidae) in field cages in Hawaii and Guatemala. Ann. Entomol. Soc. Am. 93: 1179–1185.

McInnis, D. O., T. E. Shelly, and J. Komatsu. 2002. Improving male mating competitiveness and survival in the field for medfly, Ceratitis capitata (Diptera: Tephritidae) SIT programs. Genetica 116: 117–124.

McInnis, D. O., D. R. Lance, and C. G. Jackson. 1996. Behavioral resistance to the sterile insect technique by Mediterranean fruit flies (Diptera: Tephritidae) in Hawaii. Ann. Entomol. Soc. Am. 89: 739–744.

McInnis, D. O., S.Y.T. Tam, C. Grace, and D. Miyashita. 1994. Population suppression and sterility rates induced by variable sex-ratio, sterile insect releases of *Ceratitis capitata* (Diptera: Tephritidae) in Hawaii. Ann. Entomol. Soc. Am. 87: 231–240.

Rendon, P., D. McInnis, D. Lance, and J. Stewart. 2004. Medfly (Diptera: Tephritidae) genetic sexing: large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. J. Econ. Entomol. 97: 1547–1553.

Rhode, R. H., J. Simon, A. Perdomo, J. Gutierrez, C. F. Dowling, and D. A. Lindquist. 1971. Application of the sterile release technique in Mediterranean fruit fly suppression. J. Econ. Entomol. 64: 708–713.

Rossler, Y. 1975. The ability to inseminate: a comparison between laboratory-reared and field populations of the Mediterranean fruit fly (*Ceratitis capitata*). Entomol. Exp. Appl. 18: 255–260.

Saul, S. H., and S. D. McCombs. 1993. Dynamics of sperm use in the Mediterranean fruit fly (Diptera: Tephritidae): reproductive fitness of multiple-mated females and sequentially mated males. Ann. Entomol. Soc. Am. 86: 198–202.

Severin, H. P., and W. J. Hartung. 1912. The flight of two thousand marked male Mediterranean fruit flies (*Ceratitis capitata* Wied.). Ann. Entomol. Soc. Am. 5: 400–407.

Shelly, T. E. 2001. Exposure to a-copaene and a-copaene-containing oils enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 497–502.

Shelly, T. E., T. C. Holler, and J. L. Stewart. 2006. Mating competitiveness of mass-reared males of the Mediterranean fruit fly (Diptera: Tephritidae) from eclosion towers. Fla. Entomol. 89: 380–387.

Shelly, T. E., D. O. McInnis, and P. Rendon. 2005. The sterile insect technique and the Mediterranean fruit fly: assessing the utility of aromatherapy in large field enclosures. Entomol. Exp. Appl. 116: 199–208.

Shelly, T. E., D. O. McInnis, E. Pahio, and J. Edu. 2004a. Aromatherapy in the Mediterranean fruit fly (Diptera: Tephritidae): sterile males exposed to ginger root oil in prerelease storage boxes display increased mating competitiveness in field-cage trials. J. Econ. Entomol. 97: 846–853.

Shelly, T. E., J. Edu, and E. Pahio. 2004b. Sterile males of the Mediterranean fruit fly exposed to ginger root oil induce female remating: implications for the sterile insect technique. Fla. Entomol. 87: 628–629.

Shelly, T. E., T. S. Whittier, and K. Y. Kaneshiro. 1994. Sterile insect release and the natural mating system of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 87: 470–481.

Shelly, T. E., and D. O. McInnis. 2003. Influence of adult diet on the mating success and survival of male Mediterranean fruit flies (Diptera: Tephritidae) from two mass-rearing strains on field-caged host trees. Fla. Entomol. 86: 340–344.

Shelly, T. E., and S. Kennelly. 2002. Influence of male diet on male mating success and longevity and female remating in the Mediterranean fruit fly (Diptera: Tephritidae) under laboratory conditions. Fla. Entomol. 85: 572–579.

Shelly, T. E., and D. O. McInnis. 2001. Exposure to ginger root oil enhances mating success of irradiated, mass-reared males of Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 94: 1413–1418.

Tanaka, N., L. F. Steiner, K. Ohinata, and R. Okamoto. 1969. Low-cost larval rearing medium for mass-production of oriental and Mediterranean fruit flies. J. Econ. Entomol. 62: 967–968.

Whittier, T. S., K. Y. Kaneshiro, and L. D. Prescott. 1992. Mating behavior of Mediterranean fruit flies (Diptera: Tephritidae) in a natural environment. Ann. Entomol. Soc. Am. 85: 214–218.

Whittier, T. S., F. Y. Nam, T. E. Shelly, and K. Y. Kaneshiro. 1994. Male courtship success and female discrimination in the Mediterranean fruit fly (Diptera: Tephritidae). J. Insect Behav. 7: 159–170.

Wong, T.T.Y., R. M. Kobayashi, and D. O. McInnis. 1986. Mediterranean fruit fly (Diptera: Tephritidae): methods of assessing the effectiveness of sterile insect releases. J. Econ. Entomol. 79: 1501–1506.

Wong, T.T.Y., L. C. Whitehand, R. M. Kobayashi, K. Ohinata, N. Tanaka, and E. J. Harris. 1982. Mediterranean fruit fly: dispersal of wild and irradiated and untreated laboratory-reared males. Environ. Entomol. 11: 339–343.

Zar, J. H. 1996. Biostatistical analysis, 3rd ed. Prentice-Hall, Upper Saddle River, NJ.

Received 18 May 2006; accepted 16 October 2006.